ANESTHESIOLOGY

Aquaporin 5 –1364A/C Promoter Polymorphism Is Associated with Pulmonary Inflammation and Survival in Acute Respiratory Distress Syndrome

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ANESTHESIOLOGY 2019; 130:404–13

ABSTRACT

Background: The aquaporin-5 (*AQP5*) –1364A/C promoter single-nucleotide polymorphism is associated with an altered *AQP5* expression and mortality in sepsis. Because *AQP5* expression alters neutrophil cell migration, it could affect pulmonary inflammation and survival in bacterially evoked acute respiratory distress syndrome. Accordingly, the authors tested the hypotheses that the AC/CC genotype in patients with bacterially evoked pneumonia resulting in acute respiratory distress syndrome is associated with (1) attenuated pulmonary inflammation and (2) higher 30-day survival.

Methods: In this prospective, observational study, bronchoalveolar lavage and blood sampling were performed within 24 h of intensive care unit admission. In 136 Caucasian patients with bacterially evoked acute respiratory distress syndrome, genotype of the *AQP5* –1364A/C promoter polymorphism, bronchoalveolar lavage total protein, albumin, white cell concentrations, and lactate dehydrogenase activity were measured to evaluate the relationship between genotypes and survival.

Results: AC/CC patients as well as survivors showed lower bronchoalveolar lavage protein (0.9 mg/ml *vs.* 2.3 mg/ml, $P < 0.001$ and 1.6 mg/ml *vs.* 2.6 mg/ml, $P = 0.035$), albumin (0.2 mg/ml *vs.* 0.6 mg/ml, $P = 0.019$ and 0.3 mg/ml *vs.* 0.6 mg/ml, $P = 0.028$), leukocytes (424 /ml *vs.* 1,430/ml; $P = 0.016$ and 768 /ml *vs.* 1,826/ml; $P = 0.025$), and lactate dehydrogenase activity (82 U/l *vs.* 232 U/l; $P = 0.006$ and 123 U/l *vs.* 303 U/l; $P = 0.020$). Thirty-day survival was associated with *AQP5* –1364A/C genotypes ($P = 0.005$), with survival of 62% for AA genotypes (58 of 93) but 86% for C-allele carriers (37 of 43). Furthermore, multiple proportional hazard analysis revealed the AA genotype was at high risk for death within 30 days (hazard ratio, 3.53; 95% CI, 1.38 to 9.07; $P = 0.009$).

Conclusions: In acute respiratory distress syndrome attributable to bacterial pneumonia, the C-allele of the *AQP5* –1364A/C promoter polymorphism is associated with an attenuated pulmonary inflammation and higher 30-day survival. Thus, the *AQP5* genotype impacts on inflammation and prognosis in acute respiratory distress syndrome.

(ANESTHESIOLOGY 2019; 130:404–13)

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Acute respiratory distress syndrome is defined according to clinical criteria, but lack of precise characterization may contribute to negative trials and impede personalized care. Polymorphisms of aquaporin-5, a key mediator of inflammation, may impact outcome.

What This Article Tells Us That Is New

- In acute respiratory distress syndrome attributable to bacterial pneumonia, the C-allele of the aquaporin-5 –1364A/C promoter polymorphism is associated with less pulmonary inflammation and greater survival. This may improve characterization of acute respiratory distress syndrome and ultimately facilitate individualized care.

Acute respiratory distress syndrome (ARDS) is characterized by an inflammatory destruction of pulmonary parenchymal integrity and remains an important cause of death.[1,2] However, because of advanced treatment options including maintenance of gas exchange by extracorporeal membrane oxygenation, many patients do not die from hypoxemia during early ARDS but rather from inflammation triggering maladaptive lung repair,[3] progression of lung injury to fibrosis,[4] or septic multiorgan failure.

However, wide variability exits regarding severity of lung inflammation and outcome in ARDS that cannot be explained by patients' comorbidities. Some of this variability may be influenced by genetic variations. A potential candidate for investigation is the gene encoding aquaporin-5 (*AQP5*).[5] *AQP5* mediates key mechanisms of inflammation that prevail in sepsis, including cell migration and proliferation,[6,7] activity of the renin–angiotensin–aldosterone system,[8] and the transport of water across biologic membranes.[9] Previously, we described a novel, functional, and common single-nucleotide polymorphism in the *AQP5* gene promoter (–1364A/C, rs3759129).[8] Substitution of C for A at position –1,364 was associated

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Submitted for publication May 13, 2018. Accepted for publication November 13, 2018. From the Department of Anesthesia, Intensive Care Medicine, and Pain Therapy, University Hospital Knappschaftskrankenhaus Bochum, Bochum, Germany (T.R., K.R., M.A.); and Department of Anesthesia and Intensive Care Medicine, University Duisburg-Essen and University Hospital Essen, Essen, Germany (J.P., M.A.).

with lower *AQP5* messenger RNA and *AQP5* protein expression. Furthermore, this *AQP5* promoter polymorphism is a strong and independent prognostic factor for 30-day mortality in patients with severe sepsis[10] and impacts on pathophysiologic key mechanisms.[11–13] In this context, in a lipopolysaccharide sepsis model in mice, *AQP5* expression impacted on neutrophil cell migration into the lungs[7] involving transient formation of membrane protrusions (lamellipodia and membrane ruffles) at the leading edge of the migrating cell.[6] Furthermore, target-oriented migration of neutrophils was slower and occurred to a lesser extent in humans with the AC/CC genotype of the *AQP5* −1364A/C promoter single-nucleotide polymorphism [7] Taken together, the C-allele of the *AQP5* −1364A/C promoter single-nucleotide polymorphism is associated with lower *AQP5* expression,[8] attenuated neutrophil cell migration into different organs after lipopolysaccharide administration,[7] and a lower mortality in patients with severe sepsis.[10] Therefore, the *AQP5* genotype might be a key player for pulmonary inflammation and survival in patients experiencing severe bacterial pneumonia evoking ARDS. Accordingly, we tested the hypothesis that the AC/CC genotype of the *AQP5* −1364A/C promoter single-nucleotide polymorphism in patients with bacterial pneumonia evoking ARDS is associated with (1) an attenuated pulmonary inflammation, as demonstrated by bronchoalveolar lavage characteristics, and (2) higher 30-day survival.

Materials and Methods

Patients

This study was reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Duisburg-Essen (Essen, Germany; protocol No. 01-97-1697), and written informed consent was obtained from patients or their guardians. Patients admitted to the intensive care unit of University of Duisburg-Essen Medical School (Essen, Germany) between 2009 and 2014 were considered eligible if they fulfilled the joint American/European Consensus Committee criteria for ARDS,[14] had no previous history of ARDS, and did not suffer from any type of malignant pulmonary disease. In addition, we tested all patients if they fulfilled the formal criteria of the current ARDS definition,[1,2] and no patients had to be excluded. In total, 136 patients with ARDS (79 males [58%], 57 females [42%], mean age ± SD: 43.7 yr ± 15.1) were included and studied prospectively. All patients were Germans of Caucasian ethnicity. Most patients were referred from other intensive care units for possible extracorporeal membrane oxygenation therapy after a rapidly progressive ARDS course. ARDS was evoked in 110 cases (81%) directly by bacterial pneumonia and in 26 cases (19%) by a primary extrapulmonary sepsis with a secondary bacterial pneumonia leading to ARDS. Bacterial infection was proven by positive specimen from the lung or blood culture.

Bronchoalveolar lavage and blood samples were performed within the first 24 h of intensive care unit admission. Clinical and demographic data upon study entry, including preexisting morbidities, Lung Injury Score, Simplified Acute Physiology Score II, Sepsis-related Organ Failure Assessment Score, body mass index, necessity for continuous hemofiltration/dialysis, settings of mechanical ventilation, Pao_2/fractional inspired oxygen tension ratio (Horowitz index), establishment of extracorporeal membrane oxygenation therapy, pulmonary function variables (mean airway pressure, positive end-expiratory pressure, compliance, pulmonary artery pressure, pulmonary vascular resistance), medications and dosages of vasoactive drugs, and blood chemistry values were recorded. Patients were treated using a multimodal concept that included analgesia and sedation, fluid administration, and lung-protective mechanical ventilation, anticoagulation, as well as hemodynamic, antibiotic, and diagnostic management as described previously.[15] Continuous hemofiltration/dialysis, as required, was technically performed by the hospital's nephrology department, according to standardized protocols.

The observation period was defined from admission to our intensive care unit either to day 30 of hospital stay or death. The ARDS patients were assigned to two groups (AA genotype *vs.* AC/CC genotype) depending on the −1364A/C polymorphism in the *AQP5* gene promoter. Clinical characteristics of the patient cohort are presented in table 1.

DNA Genotyping

DNA was extracted from whole blood using the QIAamp kit (Qiagen, Germany). For genotyping the single-nucleotide polymorphism −1364A/C in the *AQP5* promoter, polymerase chain reaction was performed with the forward AQP5-SE 5′–GAAACTGCAGGATGAGAGAAAT-3′, and the biotinylated reverse AQP5-AS 5′–TCTCTGTTCTCCACCTCTCCA-3′ followed by pyrosequencing as described previously.[8,10]

Bronchoalveolar Lavage

Within 24 h of admission, all ARDS patients underwent bronchoalveolar lavage for routine workup of bacterial and viral infections according to the American Thoracic Society protocol, as described previously.[16] Four aliquots of warm (37°C) sterile isotonic saline (40 ml) were instilled *via* a bronchoscope wedged into a segmental bronchus and gently withdrawn. A volume of more than 50% was recovered, filtered through cotton gauze, and centrifuged (500*g*, 10 min). The bronchoalveolar lavage supernatant was immediately frozen using liquid nitrogen, stored at −80°C, and served as a sample of the extracellular alveolar fluid.

Table 1. Characteristics of Patients (n = 136) with ARDS at Intensive Care Unit Admission as Stratified by *AQP5* –1364A/C Genotypes

Variable	AA n = 93 (68%)	AC/CC n = 43 (32%)	*P* Value	SMD
Age, yr (range/± SD)	43.9 (18–77/±15.8)	43.4 (18–66/±13.8)	0.866	–0.033
Male sex	55 (59%)	24 (56%)	0.715	–0.075
Body mass index, kg/m²	26.7 ± 6.6	25.9 ± 6.2	0.558	–0.124
Etiology of ARDS			0.908	0.152
Primary pneumonia	77 (83%)	33 (77%)		
Gram (–)	–50	–20		
Gram (+)	–18	–8		
Mixed	–9	–5		
Sepsis with secondary pneumonia	16 (17%)	10 (23%)		
Gram (–)	–9	–7		
Gram (+)	–5	–2		
Mixed	–2	–1		
Medical history, n (%)			0.867	–0.169
Previous cardiovascular disease	16 (17%)	10 (23%)		
Previous lung disease	53 (57%)	25 (58%)		
Previous gastrointestinal disease	10 (11%)	3 (7%)		
Previous renal/urogenital disease	6 (6%)	2 (5%)		
History of malignant disease	8 (9%)	3 (7%)		
Vasopressor support, n (%)	79 (85%)	34 (79%)	0.395	–0.221
ECMO therapy, n (%)	23 (25%)	14 (33%)	0.340	0.212
Continuous hemofiltration/dialysis, n (%)	47 (51%)	18 (42%)	0.346	–0.193
C-reactive protein concentration, mg/dl	20.3 [14.8–27.4]	18.5 [8,5-23.8]	0.091	–0.381
Procalcitonin concentration, ng/ml	10.4 [1.0–55.5]	5.0 [1.0–29.0]	0.324	–0.402
Leukocyte concentration, *10⁹/l	14.0 [9.0–23.1]	16.1 [11.4–23.3]	0.283	0.135
Total bilirubin concentration, mg/dl	1.0 [0.6–2.0]	0.9 [0.5–1.8]	0.633	–0.131
International normalized ratio	1.2 [1.0–1.6]	1.3 [1.0–1.6]	0.913	0.032
Platelet concentration, /nl	111 [49–228]	99 [69.5–231.5]	0.244	0.211
Partial thromboplastin time, s	46 [36–75]	47 [38–77]	0.705	0.037
Mean pulmonary artery pressure, mm Hg	34 [29–39]	38 [29–42]	0.800	0.055
PVRI, dyn*s/cm⁵*m²	276 [196–372]	303 [244.5–510]	0.696	0.007
Mean airway pressure, mm Hg	28 [24–31]	27 [22–31]	0.441	–0.165
Duration of mechanical ventilation, days	14.6 ± 9.1	16.4 ± 8.3	0.271	0.203
Ventilator free days, d	9.5 ± 9.6	10.7 ± 9.0	0.504	0.127
Horowitz-index upon intensive care unit admission	90 [68–138]	112 [84–179]	0.124	0.106
PEEP/lower airway pressure, mbar	18 [15–20]	17 [12–20]	0.343	–0.144
Simplified Acute Physiology Score II	51.7 ± 17.2	49.9 ± 15.9	0.566	–0.107
Lung Injury Score	3.3 ± 0.53	3.3 ± 0.50	0.867	±0.000
SOFA score	13.4 ± 4.9	13.8 ± 6.1	0.716	0.075

The data are presented as n (%); mean ± SD, median [25th, 75th percentile], standardized mean difference (SMD). The following missing data were excluded from the analysis: 1 case missing for body mass index; 8 cases missing for C-reactive protein concentration; 1 case missing for total bilirubin concentration; 23 cases missing for procalcitonin concentration; 12 cases missing for leukocyte concentration; 36 cases missing for pulmonary vascular resistance index; 11 cases missing for mean airway pressure; 7 cases missing for positive end-expiratory pressure (PEEP)/lower airway pressure; and 4 cases missing data for Lung Injury Score. ARDS, acute respiratory distress syndrome; ECMO, extracorporeal membrane oxygenation; Horowitz index, $Pa0_2$/fractional inspired oxygen tension; PEEP, positive end-expiratory pressure; PVRI, pulmonary vascular resistance index; SOFA, Sepsis-related Organ Failure Assessment Score.

Bronchoalveolar Lavage Leukocyte and Cytokine Concentration

In the pellet, white cell counts were assessed by counting an aliquot in a Neubauer chamber. For cell differentiation, smears were air-dried and stained according to May–Grünwald–Giemsa, as described previously.[16] Measurements of cytokine concentrations in the bronchoalveolar lavage were performed with a Procarta Cytokine assay kit (Panomics, USA) according to the manufacturer's instruction using a Luminex 200 instrument and the Luminex IS software (Luminex Corp., USA).

Albumin and Total Protein Concentrations in Bronchoalveolar Lavage

Albumin concentration in bronchoalveolar lavage supernatant was measured using an albumin enzyme-linked immunosorbent assay kit (Dade Behring, Germany). The detection limit was 1.18 μg/ml. Total protein concentration was determined after trichloroacetic acid precipitation (5%), washing, and resolubilization according to Lowry using an autoanalyzer (Technicon, USA) and bovine serum albumin as standard.

Bronchoalveolar Lavage Lactate Dehydrogenase Activity

Total lactate dehydrogenase activity (LDH1–LDH5) in bronchoalveolar lavage supernatant was measured by a kinetic ultraviolet test (Diaglobal GmbH, Germany) using an optimized standard method (International Federation of Clinical Chemistry and Laboratory Medicine).

Chemicals

All chemicals were of highest available or analytical grade. Water was deionized, distilled, and passed through a Milli-Q system (Millipore, Germany) before use.

Statistical Analysis

This is the primary analysis of these data, and the statistical approach was mostly designed *a priori* (table 1, table 2, and figs. 1–3). Only the analysis provided in table 3, Supplemental Digital Content 1–4 (http://links.lww.com/ALN/B824, http://links.lww.com/ALN/B825, http://links.lww.com/ALN/B826, http://links.lww.com/ALN/B827), and the evaluation of the formal effect size in table 1 arose out of the review process and were thus designed *post hoc*. Continuous variables are presented as means ± SD in case of normal distribution and as median and interquartile range (25^{th}; 75^{th} percentile) in case of nonnormally distributed variables. Categorical variables were characterized by numbers with percentage and were compared using the chi-square test. Continuous variables were compared using independent *t* test or nonparametric Wilcoxon Mann–Whitney test. Potential outliers were evaluated using boxplots, but no action was necessary. Although no statistical power calculation was conducted before this observational study, the number of patients was based on our previous experience with sepsis patients.[10] In this context, we presented the formal effect size as the standardized mean difference or the mean difference between the groups with the 95% CI applying bootstrap resampling. The *AQP5* –1364A/C single-nucleotide polymorphism distributions were tested for deviations from the Hardy–Weinberg equilibrium (exact two-sided *P* value, significance value 0.05). Explorative comparisons by *AQP5* –1364A/C genotypes (AC/CC *vs.* AA) were performed for several clinical patient characteristics (table 1). AC and CC were combined because of the low frequency (3.7%, 5 of 136) of the CC genotype, referring to a dominant model with "A" as risk allele and "C" as a protective or low-risk allele. All characteristics of the AA, AC, and CC genotypes were additionally compared for a gene dose effect, as shown in Supplemental Digital Content 1–4 (http://links.lww.com/ALN/B824, http://links.lww.com/ALN/B825, http://links.lww.com/ALN/B826, http://links.lww.com/ALN/B827).

The clinical endpoint was 30-day survival dependent on *AQP5* –1364A/C genotype. Survival probabilities were graphically assessed by the Kaplan–Meier method. The log-rank test was used to evaluate the univariate relationship between *AQP5* –1364A/C genotype and clinical outcomes. Afterward, we performed Cox regression analyses assessing the joint impact of *AQP5* –1364A/C genotype, as well as of potential predictors for survival. At first, Cox regression was performed with several models with a single predictor. Thereafter, multiple variable Cox regression was performed with an initial model investigating multiple predictors simultaneously. To avoid overfitting, a restricted model with only three predictors was assessed afterward only using those predictors with a *P* value of 0.05 or lower in either the single or the multiple predictors comparisons (table 2). Furthermore, no attempt was made to adjust for multiplicity. CIs were calculated with coverage of 95%. All reported *P* values are nominal and two-sided with an *a priori* α error of less than 0.05. All analyses were performed using SPSS (version 24, IBM, USA), and for graphical presentations GraphPad Prism 7 (Graph-Pad, USA) was used.

Results

The observed 30-day survival of the entire cohort was 70% (95 of 136), and the median duration of intensive care unit stay was 21 days (11; 34 days). Table 1 shows genotype frequencies and the characteristics of the 136 ARDS patients upon admission and grouped by their single-nucleotide polymorphism of *AQP*5 promoter. Regarding distribution of the genetic variations of the *AQP5* single-nucleotide polymorphisms, we observed a frequency of 93 for the AA

Table 2. Cox Regression Analysis in Patients with Acute Respiratory Distress Syndrome (n = 136)

	Single Predictor		Multiple Predictors			
			Initial		Restricted	
Covariable	Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
Aquaporin-5 –1364A/C genotype						
AC/CC	1	—	1	—	1	—
AA	3.72 (1.43–9.71)	0.007	3.23 (1.11–9.93)	0.031	3.53 (1.38–9.07)	0.009
Sex						
Women	1	—	1	—		
Men	0.97 (0.56–2.21)	0.654	1.39 (0.61–3.16)	0.429		
Age, yr	1.01 (0.98–1.03)	0.531	1.01 (0.98–1.03)	0.614		
Body mass index, kg/m²*	0.93 (0.87–1.00)	0.059	0.92 (0.83–1.02)	0.101		
SAPS II (per unit)	1.04 (1.01–1.06)	0.006	1.04 (1.01–1.06)	0.008	1.02 (1.01–1.04)	0.012
Pao_2/Fio_2*	1.00 (0.99–1.00)	0.193	1.00 (0.99–1.00)	0.161		
Dialysis						
No	1	—	1	—		
Yes	1.37 (0.66–2.87)	0.398	1.21 (0.48–3.08)	0.682		
ECMO						
No	1	—	1	—		
Yes	1.16 (0.51–2.61)	0.723	0.77 (0.29–2.10)	0.618		
C-reactive protein concentration, mg/dl†	0.99 (0.96–1.02)	0.542	0.99 (0.96–1.01)	0.299		
Total serum bilirubin concentration, mg/dl*	1.31 (1.08–1.59)	0.006	1.19 (1.00–1.41)	0.050	1.13 (1.04–1.24)	0.006
Platelet concentration, 10^9/l‡	0.99 (0.99–1.00)	0.107	1.00 (0.99–1.00)	0.210		

Hazard ratio point estimates, 95% CI, and *P* values (two-sided) are reported. Missing data were right censored as explained in notes below. Homer–Lemeshow statistics were as follows: $\kappa^2 = 7.236$; $P = 0.511$. *One case missing; †Eight cases missing; ‡Seven cases missing. ECMO, extracorporeal membrane oxygenation; SAPS II, Simplified Acute Physiology Score.

genotype (expected: n = 92.2), 38 for the AC genotype (expected: n = 39.5), and 5 for the CC genotype (expected: n = 4.2) in our cohort. Accordingly, no deviation from the Hardy–Weinberg equilibrium was observed ($P = 0.655$). We found no evidence for statistically significant associations of *AQP5* –1364A/C genotypes with age ($P = 0.866$, standardized mean difference = –0.033), sex ($P = 0.715$, standardized mean difference = –0.075), body mass index ($P = 0.558$, standardized mean difference = –0.124), necessity for extracorporeal membrane oxygenation therapy ($P = 0.340$, standardized mean difference = 0.212), continuous hemofiltration/dialysis ($P = 0.346$, standardized mean difference = –0.193), Simplified Acute Physiology Score II ($P = 0.566$, standardized mean difference = –0.107), or Sequential Organ Failure Assessment score ($P = 0.716$, standardized mean difference = 0.075; table 1). Moreover, there was no genotype-dependent pattern for infection type ($P = 0.495$, standardized mean difference = 0.152), primary diagnosis at hospital admission ($P = 0.867$, standardized mean difference = –0.169), duration of mechanical ventilation ($P = 0.271$, standardized mean difference = 0.203), ventilator-free days ($P = 0.504$, standardized mean difference = 0.127), or the Horowitz index upon intensive care unit admission ($P = 0.124$, standardized mean difference = 0.106; table 1).

Total protein concentration in bronchoalveolar lavage supernatant was 2.4-fold ($P < 0.001$, standardized mean difference = –0.917), albumin concentration 2.4-fold ($P = 0.019$, standardized mean difference = –0.652), lactate dehydrogenase activity 2.8-fold ($P = 0.006$, standardized mean difference = –0.908), and leukocyte concentration 3.4-fold ($P = 0.016$, $P = –0.698$) lower in C-allele carriers compared with AA genotype patients (fig. 1). In contrast to values of bronchoalveolar lavage variables, we found no statistically significant associations of *AQP5* –1364A/C genotypes with serum total protein ($P = 0.191$, standardized mean difference = –0.399), serum albumin ($P = 0.827$,

Table 3. Comparison of Plasma Cytokine Concentrations, BAL Cytokine Concentrations, and BAL Leukocyte Subtypes among Genotypes of the *AQP5* –1364A/C Promoter Single-nucleotide Polymorphism

	AA Genotype			AC/CC Genotype				
Cytokine Concentration	**Median**	**[25th; 75th]**	**n**	**Median**	**[25th; 75th]**	**n**	***P* Value**	**MD (95% CI)**
Serum								
TNF-α, pg/ml	4.3	[2.1; 8.7]	54	3.9	[2.2; 6.1]	27	0.205	2.4 (–0.5 to 5.5)
Interleukin 6, pg/ml	1,750	[694; 3,717]	61	1,485	[458; 2,415]	30	0.169	882 (–462 to 2,227)
Interleukin 10, pg/ml	9.4	[1.6; 68.9]	61	6.0	[2.5; 48.9]	30	0.513	4.7 (–54.9 to 54.4)
BAL								
TNF-α, pg/ml	9.9	[6.5; 15.5]	59	8.3	[6.5; 12.3]	28	0.145	3.1 (0.3 to 5.9)
Interleukin 6, pg/ml	681	[186; 1,310]	59	329	[198; 611]	28	0.018	658 (259 to 1,089)
Interleukin 10, pg/ml	10.9	[2.9; 71.5]	59	5.5	[3.4; 11.3]	28	0.129	6.9 (2.1 to 13.1)
Neutrophil count, ml^{-1}	336	[118; 1,215]	48	142	[47; 226.2]	19	0.005	856 (371 to 1,406)
% Neutrophils, 95% CI	65.9	57.8 to 73.6		54.8	42.0 to 66.5			
Macrophage count, ml^{-1}	131	[44; 370]	48	113.2	[35; 221]	19	0.412	173 (31 to 331)
% Macrophages, 95% CI	29.3	21.3 to 37.9		39.2	26.9 to 52.3			
Lymphocyte count, ml^{-1}	9	[0; 31]	48	7	[3; 14]	19	0.714	11 (–18 to 35)
% Lymphocytes, 95% CI	3.8	2.2 to 5.4		5.0	2.4 to 8.9			

Median with corresponding interquartile range [25th; 75th], mean difference (MD) with 95% CI, number of patients (n), and *P* values (Mann–Whitney *U*) or 95% CI are reported. BAL, bronchoalveolar lavage; TNF, tumor necrosis factor.

standardized mean difference = –0.110), lactate dehydrogenase activity (P = 0.785, standardized mean difference = –0.101), or leukocyte concentrations, respectively (P = 0.283, standardized mean difference = 0.135; fig. 1). Additionally, AC/CC genotypes showed both lesser interleukin 6 concentrations (P = 0.018, mean difference = 658, 95% CI, 259 to 1,089) and lower neutrophil counts (P = 0.005, mean difference = 856, 95% CI, 371 to 1,406) in bronchoalveolar lavage fluid compared with AA genotypes (table 3).

Bronchoalveolar lavage total protein concentration (1.7-fold; P = 0.035, standardized mean difference = 0.435), albumin concentration (1.7-fold; P = 0.028, standardized mean difference = 0.529), lactate dehydrogenase activity (2.5-fold; P = 0.020, standardized mean difference = 0.770), and white cell concentration (2.4-fold; P = 0.025, standardized mean difference = 0.555) were all greater in ARDS nonsurvivors compared with survivors (fig. 2).

Thirty-day survival was statistically significant associated with *AQP5* –1364A/C genotypes (P = 0.005; fig. 3). Thirty-day survival was 62% for AA genotypes (58 of 93) and 86% for AC/CC genotypes (AC 33 of 38; CC 4 of 5). Cox regression analyses revealed the *AQP5* –1364A/C genotype status both as a strong as well as an independent prognostic factor when jointly considering other predictors of 30-day survival. Homozygous AA subjects were at high risk of death within the 30-day observation period (hazard ratio, 3.53, 95% CI, 1.38 to 9.07; P = 0.009; table 2). Additionally, Cox regression revealed total serum bilirubin concentration (hazard ratio, 1.13; 95% CI, 1.04 to 1.24; P = 0.006) and the Simplified Acute Physiology Score II (hazard ratio, 1.02; 95% CI, 1.01 to 1.04; P = 0.012) as further independent risk factors within the 5% significance level (table 2).

Discussion

This study shows that the AA genotype of the *AQP5* –1364A/C polymorphism is associated with aggravated pulmonary inflammation, as suggested by higher bronchoalveolar lavage protein and leukocyte concentrations, as well as lactate dehydrogenase activity, and carries a substantially higher 30-day mortality in patients with bacterially evoked ARDS. Furthermore, the AA genotype is an independent prognostic factor for 30-day mortality with an estimated hazard ratio of 3.53.

The exact mechanisms of genotype-related death with the AA and AC/CC genotype cannot be pinpointed by our study (*e.g.*, because of the absence of pulmonary biopsies and quantitative histochemistry). However, based on our bronchoalveolar lavage data, an altered pulmonary inflammation is the likely cause, as suggested by bronchoalveolar lavage abnormalities, and this hypothesis is also supported by previous evidence.[7,12,13] In this context, wild-type mice showed both an aggravated migration of neutrophil

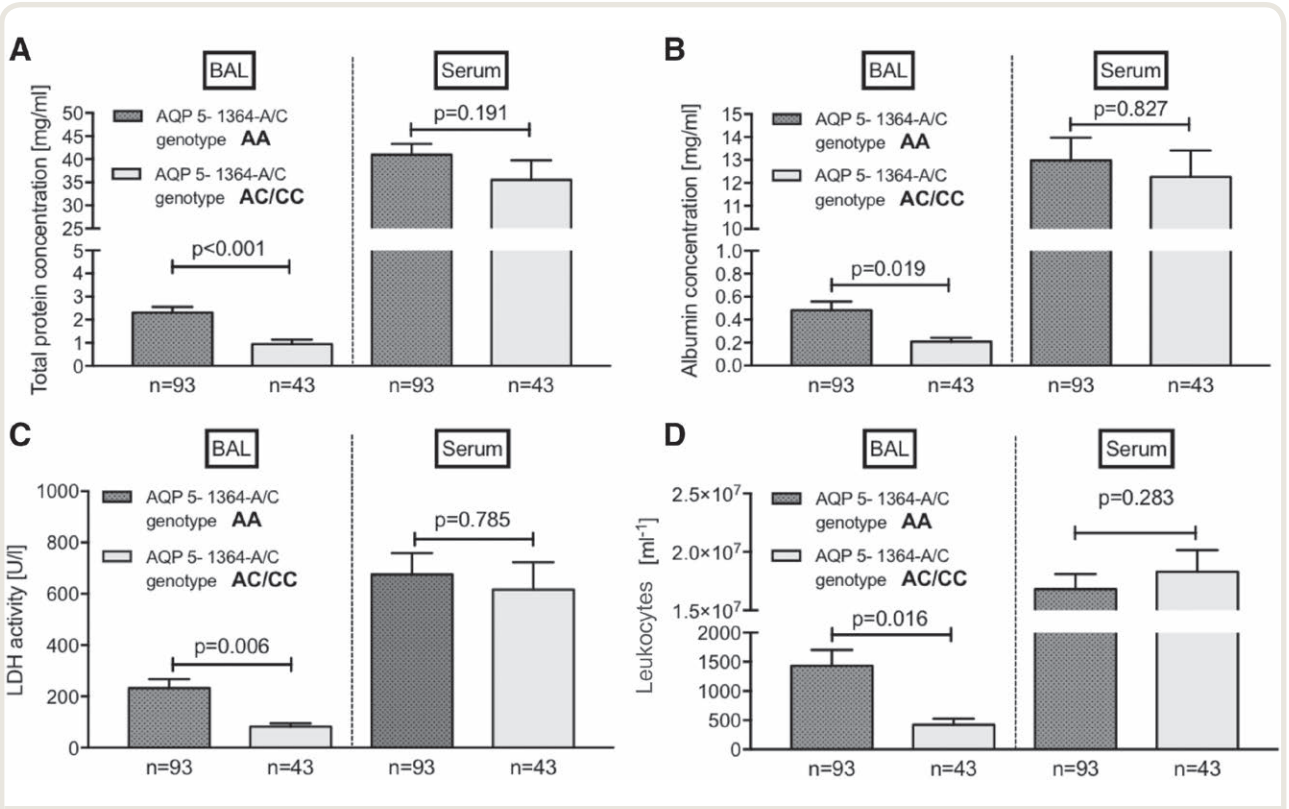

Fig. 1. Bronchoalveolar lavage (BAL) and serum measurements stratified for the aquaporin-5 (*AQP5*) –1364A/C promoter genotype in patients with acute respiratory distress syndrome (ARDS). Each *first column* indicates patients with the homozygous AA genotype, and each *second column* indicates carriers of the C-allele (AC and CC genotypes). The AC/CC genotype shows lower BAL total protein concentrations (*A*), albumin concentrations (*B*), lactate dehydrogenase (LDH) activity (*C*), and white cell concentration (*D*), suggesting attenuated pulmonary inflammation, whereas the respective sera do not show such differences between genotypes.

granulocytes into the lung and higher mortality compared with *AQP5*-knockout mice.[7] Furthermore, target-oriented migration of human neutrophils *in vitro* is faster and occurs to a larger extent in case of higher *AQP5* expression attributable to the AA genotype of the *AQP5* –1364A/C single-nucleotide polymorphism.[7] Of interest, Zhang *et al.*[17] reported that a greater *AQP5* expression is protective in the maintenance of pulmonary barrier function in *Pseudomonas aeruginosa* evoked acute lung injury. However, in our ARDS cohort we found no differences between AA an AC/CC genotypes regarding the incidence of a *P. aeruginosa* infection on admission in bronchoalveolar lavage and blood cultures (AA n = 14; AC/CC n = 8; $P = 0.601$). Strikingly, Zhang *et al.* also showed that an *AQP5* deficiency is associated with declined activation of mitogen-activated protein kinase and nuclear factor-κB pathways. Taking these results into account, the *AQP5* –1364A/C promoter single-nucleotide polymorphism in ARDS might represent a double-edged sword. On the one hand, the AA genotype aggravates the immune cell migration to the infected tissues, which is associated with a better bacterial eradication and less bacterially evoked harm. On the other hand, exaggerated immune cell migration as observed in AA genotypes may evoke greater release of proteases and reactive oxygen species, potentially damaging the host's tissues.[18] The latter hypothesis is in line with our results demonstrating lower protein and albumin concentrations as markers for pulmonary inflammation,[19,20] as well as lower lactate dehydrogenase activity, a marker for pulmonary tissue damage,[21] in the bronchoalveolar lavage supernants of AC/CC genotypes.

Current research has also focused on the involvement of the renin–angiotensin system in the pathogenesis and clinical outcome of ARDS. Renin–angiotensin system signaling and increased concentrations of angiotensin were linked to inflammation, fibrosis, and impaired lung function and are potentially associated with a worse outcome.[22] We previously described that the *AQP5* –1364A/C single-nucleotide polymorphism alters renin–angiotensin system regulation in young and healthy humans, as well as in patients with coronary heart disease.[8] However, we also showed that the *AQP5* –1364A/C polymorphism is not associated with altered plasma angiotensin or serum aldosterone concentrations in patients experiencing severe sepsis.[10] Nevertheless, further research on this topic may offer a better understanding of the role of renin–angiotensin system and *AQP5* in ARDS pathophysiology.

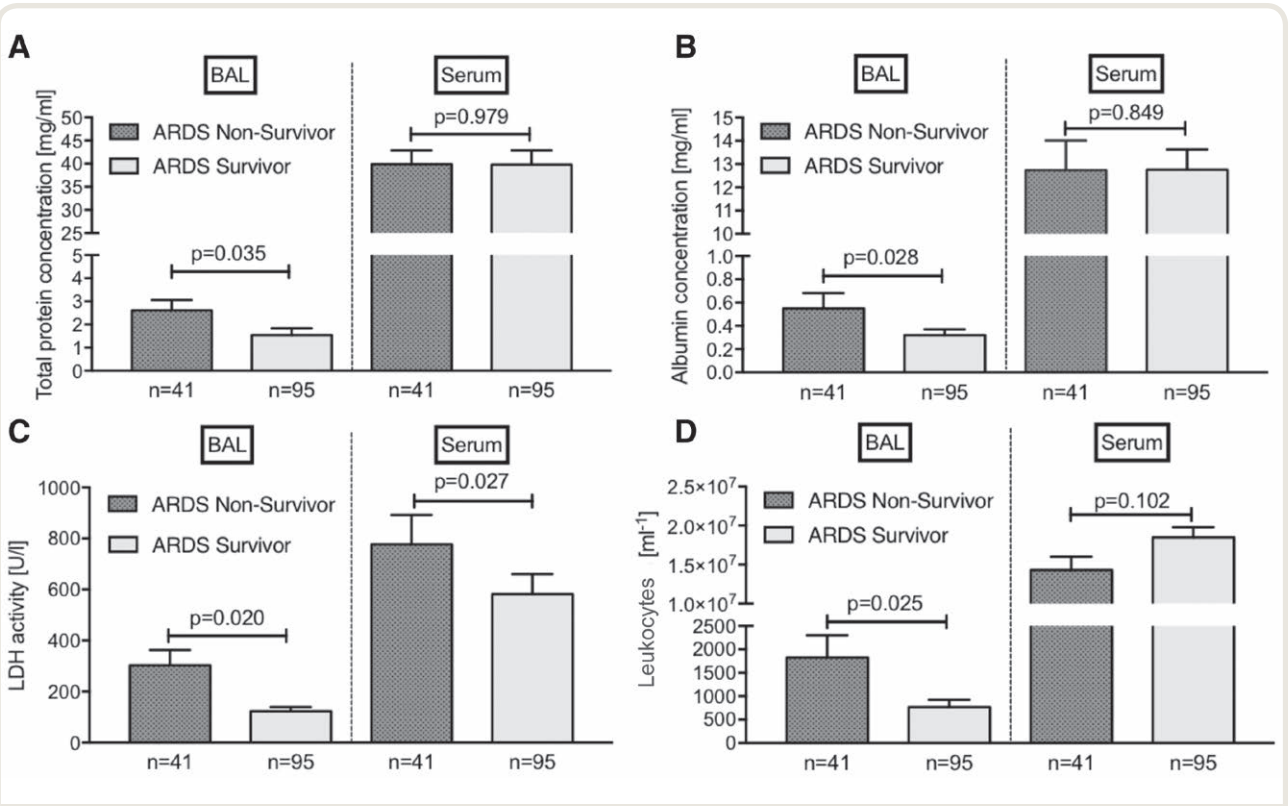

Fig. 2. Bronchoalveolar lavage (BAL) and serum measurements stratified for the aquaporin-5 (*AQP5*) –1364A/C promoter genotypes and 30-day survival. Each *first column* indicates acute respiratory distress syndrome (ARDS) nonsurvivors, and each *second column* indicates ARDS survivors. The ARDS nonsurvivors showed a higher total protein concentration (*A*), albumin concentration (*B*), lactate dehydrogenase (LDH) activity (*C*), and leukocyte concentration (*D*).

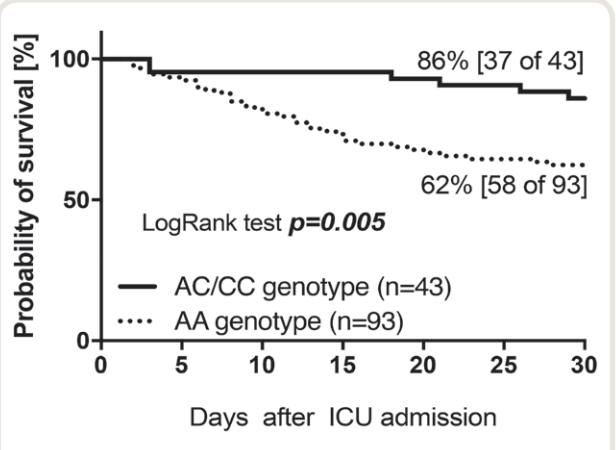

Fig. 3. Thirty-day survival in patients with acute respiratory distress syndrome. Kaplan–Meier estimates were used to calculate probabilities of 30-day survival based on aquaporin-5 –1364A/C promoter polymorphism. Thirty-day survival was higher in C-allele carriers compared with AA genotypes. ICU, intensive care unit.

Taken together, the C-allele of the *AQP5* promoter single-nucleotide polymorphism seems to be beneficial for survival in acute inflammatory diseases with an overwhelming initial immune response such as ARDS or sepsis. In this context, our prior study in a different cohort of patients with sepsis showed that the most frequent AA genotype is at high risk for death within 30 days with a hazard ratio of 3.59, strengthening our present findings.[10] Additionally, our data allow us to speculate on a gene dose effect attributable to the lower mean values of lactate dehydrogenase activity, albumin, leukocyte, and total protein concentrations in patients with the CC genotype when compared with patients with the AC genotype (Supplemental Digital Content 3, http://links.lww.com/ALN/B826). In fact, the log-rank test of our Kaplan–Meier estimates remains statistically significant after being stratified for all three groups (*i.e.*, AA, AC, and CC genotypes; $P = 0.019$; Supplemental Digital Content 4 [http://links.lww.com/ALN/B827]). However, there is no statistically significant difference between AC and CC genotypes, as expected, because of the rare occurrence of the CC genotype (n = 5). Hence, definite conclusions regarding a strict gene dose effect cannot be drawn.

In mice, lung injury is associated with downregulation of *AQP5* expression in lung tissue,[23–25] and it has been speculated that lower *AQP5* expression in the lung may disrupt the alveolar epithelial barrier worsening outcome.[26,27] Other

studies in mice, however, do not support that view,[10,28–30] and *AQP5* expression was only altered with lipopolysaccharide-mediated lung injury, but neither in HCl-evoked nor in ventilation-induced lung injury.[11] Thus, the role of *AQP5* may depend both on species differences and on the type of specific acute inflammatory response. Accordingly, it would appear prudent to sample as much data from human subjects and patients as ethically feasible. In any case, our data demonstrate a genotype-dependent effect of *APQ5* genotype both on severity of pulmonary involvement and survival in bacterially evoked ARDS.

Limitations

Unrecognized selection bias, inherent to many genetic-association studies, cannot be excluded entirely. Because our study was conducted in patients of European-Caucasian descent, findings cannot be generalized to subjects of other ancestry. Furthermore, although all patients were treated with a rather standardized multimodal regimen, undetected confounding factors may have distorted the results because of the multifactorial nature of ARDS. Moreover, we cannot exclude that different time frames of ARDS onset and its duration until referral to our intensive care unit may have influenced our results. Finally, given the complex pathophysiology of pulmonary changes in ARDS and our study design, we cannot completely exclude that *AQP5* and inflammation-independent changes may have contributed to protein and albumin accumulation in the bronchoalveolar lavage and eventually to mortality. Nevertheless, for the given entity, the study population was not small, and Cox regression analyses revealed *AQP5* –1364A/C single-nucleotide polymorphism as both a statistically significant and independent factor for 30-day survival. Furthermore, bronchoalveolar lavage composition was only measured once (within 24 h after intensive care unit admission). Although repeated measurements at fixed time intervals and lung biopsies measuring tissue *AQP5* expression may have expanded our findings, considerations regarding patients' safety are incompatible with such an approach.

Conclusions

The *AQP5* AC/CC genotype is associated with both an attenuated pulmonary inflammation and substantially higher 30-day survival in patients with bacterially evoked ARDS. Thus, *AQP5* –1364A/C promoter single-nucleotide polymorphism impacts on ARDS survival.

Acknowledgment

The authors thank Winfried Siffert, M.D., Institute of Pharmacogenetics at the University Hospital Essen, Essen, Germany, for genotyping the study patients by pyrosequencing.

Research Support

Support for this study was provided solely from institutional and/or departmental sources.

Competing Interests

The authors declare no competing interests.

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